

Feuille 1

	PCR 20 µL reaction (units : uL)	Final Concentration			Primers:
10x thermopol reaction buffer	2,5	1X			f_Gbs_CYC
10 mM dNTPs	0,5	200 µM			r_Gbs_CYC
10 µM Forward Primer	0,5	0.2 µM			
10 µM Reverse Primer	0,5	0.2 µM			
Template (colony)	0	5 ng			DNA Template:
DMSO (optional)	0	3 %			Colonies
Taq polymerase	0,125	1.0 units/50 µl PCR			
Nuclease-free Water	20,875				
Total volume	25				
Number of reactions (including controls):	6				CYC --> 5 colonies
					1 negative control
WORK ON ICE					
Mastermix				Tubes	C1
10x Thermopol reaction buffer	15				C2
10 mM dNTPs	3				C3
10 µM Forward primer	3				C4
10 µM Reverse primer	3				C5
DMSO (optional)	0				C -
Taq DNA Polymerase (ADDED LAST)	0,75				
Nuclease-free Water	125,25				
Distribute 24 µl in tubes					
Program	Y-COL-B				
PCR programming: programme B 'Y-COL-B' machin					
Initial Denaturation	95°C	7 minutes			
	95°C	30 sec			
	62°C	1 minute			
35 Cycles	67°C	1 minute 15			
Final Extension	72°C	5 minutes			
Hold	4°C	hold			